Abstracts

3045

Eastern Experiment Station Collaborators' Conference on

FOOD SAFETY

October 29 & 30
·1968·
Philadelphia,
Pennsylvania

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

This is a collection of abstracts of the papers presented before the Eastern Experiment Station Collaborators' Conference on Food Safety.

Views expressed are not necessarily those of the U. S. Department of Agriculture. Requests for further information must be sent to the speakers. Mention of commercial products or firms does not imply recommendation or endorsement by the Department of Agriculture over others not mentioned.

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INTRODUCTION

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This conference on food safety was undoubtedly one of the most significant, in terms of subject matter and information presented, of the long series of Experiment Station Collaborators' Conferences it has been our pleasure to host. When I met with the Experiment Station Directors last March, we picked this subject because we agreed that meeting the ever more stringent requirements of the Food and Drug Administration to ensure absolute safety in the food our people consume is one of the food industry's most urgent concerns.

To provide a better understanding of the subject, we brought together FDA officials with scientists in government and in the universities who are involved with the protection of the Nation's food supply. The program presented at the conference was arranged by William L. Sulzbacher and his committee consisting of Dr. Leroy C. Blankenship, Charles N. Huhtanen, and Dr. Joseph Naghski. They asked a toxicologist and a food technologist from FDA to discuss the subject broadly, and then they obtained specialists to discuss such specific subjects as toxic substances formed in food by oxidation, radionuclide contamination, food allergens, the role of animal parasites in making foods unsafe, and the whole area of microbial contamination.

The abstracts appearing in this publication should give a fair idea of the breadth of coverage given in the conference to this vital subject.

CHEMICALS IN FOODS AS VIEWED BY THE TOXICOLOGIST

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We live in an age in which man is exposed to an increasing number of new chemicals. These reach us not only through our food but also as medicines and from our environment. It is no longer possible to evaluate the safety of chemicals in food without considering the larger problem of safety of chemicals from all sources.

In testing new chemicals on laboratory animals to evaluate their safety for man several areas have been receiving increased emphasis. One of these is the investigation of possible effects on the reproduction function. Here, in addition to effects on fertility, we are concerned with teratogenic and mutagenic activity. A second area of increased interest involves the metabolic fate of the new chemical. Into what compounds is it converted? What organs and what enzyme systems participate in these changes? Which of the experimental animals most closely resemble man in their metabolism of the chemical?

With increased refinement of technique we sometimes uncover changes whose significance is not immediately apparent. Although every change is not by definition unfavorable, caution requires us to be suspicious of any chemically induced alteration in bodily structure or function. Similar caution is called for in statistical interpretation of results. The proper question is not, "has it been proven beyond all doubt that the chemical caused this effect?" but rather, "how bad could the 'true' state of affairs be and still be reasonably consistent with the results observed in this necessarily imperfect sample?"

REMOVAL OF RADIONUCLIDES FROM FOODS

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During the testing of nuclear weapons a few years ago, atmospheric fallout was found to contain several radionuclides which eventually found their way into food products. The probability that a radioactive substance may constitute a health hazard depends not only on the amount ingested, but also on the half-life of the radionuclide and on the degree to which it is concentrated and retained in certain tissues of the body. The radionuclides which concern us most from the public health standpoint are strontium-90, iodine-131, strontium-89, cesium-137, and barium-140. All of these have been found in significant amounts in fresh milk. Since milk is consumed by a large segment of the U. S. population, it is the single food most used in estimating the intake of selected radionuclides by the general population.

The effectiveness of a method to reduce the levels of radionuclides in food products depends on the chemical and biological properties of the element, and on its radioactive half-life. The use of dry feeds and the storage of milk or milk products are effective means of minimizing the levels of iodine-131 in milk, but have little value for solving the strontium-90 problem. Therefore, a process for removing strontium-90 from milk was considered a highly desirable standby countermeasure. Under some circumstances, removal of iodine-131 from milk may also be the most effective means of controlling the levels of this isotope. Such processes were developed through a research and development program supported jointly by the Atomic Energy Commission, the Public Health Service and the Agricultural Research Service. The pilot plant development was carried out at the Agricultural Research Center, Beltsville, Maryland. Commercial scale testing of the automated pilot plant process was accomplished under contract with the Producers Creamery Company, Springfield, Missouri.

The process for removing radiostrontium consists of passing milk (acidified to a pH of 5.3 with citric acid) through fixed-bed columns containing a strong acid ion-exchange resin. About 95 percent of the radioactive strontium can be removed by this process while maintaining the milk's chemical composition near normal, and inducing only small changes in its physical stability and flavor. The nutritional quality of milk processed for removal of strontium-90 by the fixed-bed method was evaluated by determining the growth rates of baby pigs and rats and by chemical analyses. Results showed no significant differences in the body weight gains between the animals fed on milk processed by ion exchange and those fed normal milk. The thiamine, niacin, and vitamin B_6 contents of the processed milk decreased 50, 27 and 15 percent, respectively. The remaining vitamins and minerals were unaffected, except for an increase in potassium and citrate which resulted from the acidification and neutralization steps involved in the removal process. Details of the pilot plant processing operations for removal of radiostrontium are described in previous papers (2,7).* Likewise, results of the nutritional evaluation of the process are discussed in previously published papers (4,5).

*Underscored numbers refer to references on next page.

A pilot plant was established for removing both iodine-131 and radiostrontium from milk by a fixed-bed ion-exchange system. The milk was first passed through an anion resin column to remove iodine-131, then acidified to pH 5.3 and passed through a cation column. More than 93 percent of the iodine-131 was removed. Details of the integrated anion-cation removal process are described in a separate paper (9). Results of the large scale (commercial) tests of the fixed-bed ion-exchange removal systems are reported in references 3, 6, and 8.

Some research has been done on removing radioisotopes from meat and other foods (1). Iodine-131 removal is best accomplished by delaying distribution until radioactive decay occurs. Cesium-137 can be leached out of meats, fruits, and vegetables. It can also be washed from the surface of fruits and vegetables.

Strontium-90 is concentrated in the outer layers of wheat; consequently, most of its activity is removed in the milling process.

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FORMATION AND DESTRUCTION OF TOXIC SUBSTANCES IN FATS BY HEAT AND OXIDATION

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About fifteen years ago, scientists became aware of the toxic properties of some of the materials formed in autoxidized fats, especially the fat polymers. Although some of their fears were unfounded, fat oxidation continues to be a subject of biological importance.

Interest has turned from the polymeric fractions (obtained from fats which had been damaged far more than would occur normally) to the relation of dietary peroxidized materials to tissue damage. The original impression that dietary peroxidized materials have little toxicity has been modified by the recognition that they may exert some harmful effects. They may damage vitamins and other dietary constituents to bring about disease (mulberry heart disease in pigs); their prolonged intake may weaken the intestinal wall to such an extent that they are absorbed.

Recent work has shed new light on the interrelationship between the dietary level of vitamin E (tocopherol) and the presence in the diet of peroxidized or unsaturated fat. Formerly, it was believed that the protective effect exerted by tocopherol was due to its antioxidant action in preventing tissue peroxidation. This could supposedly lead to the uncontrolled formation of free radicals, especially if the diet was rich in unsaturated fat. However, a group of English workers (Brit. J. Nutr., Vol. 21) has shown that vitamin E deficiency is not associated with increased tissue peroxidation. They concluded that the damaging effects of long-chain unsaturated and oxidized fatty materials are due to a direct stress on the metabolism of cells, which increases the vitamin E requirement in these cells.

These findings may have some bearing on McKay's studies on the occurrence of intravascular clotting (generalized Schwarzman reaction) in pregnant rats fed a vitamin E-low diet containing oxidized cod liver oil. When the oxidized cod liver oil was transesterified to the ethyl esters and the latter were molecularly distilled into fractions, it was found that the toxicity had been concentrated in the late-distilling fractions containing mainly long-chain unsaturated and oxidized fatty acids (C 20-24). The deficient animals having the disease were found to have high levels of these acids in their serum and low levels in the liver. The opposite was the case in the group given tocopherol and not showing the disease.

From another study, it was concluded that vitamin E prevents damage to the so-called reticuloendothelial system and thus helps clear the blood of clot-inducing materials.

Not all studies have shown that oxidation of fat is deleterious. When rats were fed corn oil, soybean oil, and olive oil which had been aerated at 60° C. for 40 hours in a long-term study, they had a significantly longer life span than did controls fed the fresh oils. Inasmuch as the oxidation did not usually result in a higher peroxide value in the oil, it was postulated that the vegetable oils contained antioxidants which were inactivated by the aeration and that these may have been the toxic substances. Such antioxidants have been found in many plants. Therefore, the unsaponifiable fraction of vegetable oils is being investigated. Such investigations may eventually necessitate modifications in present methods of processing fats for human consumption.

The examples of the mulberry heart disease and experimental eclampsia suggest studies of purified oxidized lipids in animals under stress.

THE MISSING ADDITIVE

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The chemists and food technologists engaged in research with chemicals to be used in or on foods need to understand the provisions of the Federal Food, Drug, and Cosmetic Act.

All food additives, pesticides, and color additives are subject to the pre-marketing clearance requirements of the Act administered by the Food and Drug Administration (FDA). The information necessary to be submitted to FDA for approval should be considered during the planning phases of the research work. Research planned in this manner may save money and time in providing the data which will be needed for inclusion in any future petition submitted to FDA for a regulation permitting the marketing of the chemical for use in or on food.

Failure on the part of the petitioners to understand clearly the requirements of the Act has led to the rejection by FDA of many petitions. Often this has been because of missing data which are needed to support the regulation as requested in the petition. When this occurs, money and time are wasted by both the petitioner and FDA.

To the extent possible, FDA is willing to discuss proposed protocols of planned experiments with petitioners, so long as it is understood that mere completion of the work discussed will not lead automatically to the desired regulation.

FOOD ALLERGENS

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Allergy is an immunological disease responsible for a whole group of syndromes, comprising such afflictions as hayfever, asthma, hives, gastrointestinal disturbances, certain forms of eczema, and migraine headache. Evidences of the unusual sensitivity of some individuals to common foods are found in some of the earliest records. It seems logical to assume that these disorders accompanied the evolution of the immunological defense mechanisms. Food allergy is primarily a problem in infants and children under two years of age, but also affects a few adults. The incidence of food allergy is not known, primarily because of the difficulties in diagnosing the causative factor. Not all individuals react to diagnostic skin tests, so that diagnosis is usually determined by clinical observations.

A review of the recent discoveries of the antibodies and chemical mediators in animal anaphylaxis indicates that the reactions referred to as anaphylaxis in animals are prototypes of allergic reactions in the human. The allergic antibody has recently been discovered to be associated with a previously unrecognized immunoglobulin, now designated as Immunoglobulin E. The properties of the anaphylactic antibodies of the rat, rabbit and dog are remarkably similar to the allergic antibody.

The most common food allergens are wheat, eggs, and milk, but any food is capable of being an allergen. Though less common, such foods as fish, shellfish and cottonseed produce the most dramatic clinical picture in allergy, and are most commonly associated with diagnostic skin tests.

PARASITES AND FOOD SAFETY

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That parasites of certain food-producing animals render the meat unsafe for human consumption has been known for many centuries. It is believed by parasitologists that the Mosaic Law forbidding the Jewish people to eat pork is perhaps the first indication of the practical application of this knowledge. Certain Chinese medical writings extending from 180 B.C. indicate some knowledge and opinion concerning helminths in food. However, it was not until 1684 that the animal nature of helminth larvae was recognized in Western Europe.

Some of the more important tissue parasites of animals used for human food that are known to infect man when they are ingested are as follows:

Infective stage	Source of infection	Parasite in man	Location
Tapeworms (Cestodes):			
Cysticercus bovis	Beef	Taenia saginata (beef tapeworm)	Small intestine
Cysticercus cellulosae	Pork	Taenia solium (pork tapeworm) Cysticerci (from autoinfection)	Small intestine Muscles, eye, brain
Plerocercoid	Freshwater fish	Diphyllobothrium latum	Small intestine
Plerocercoid	Amphibians; reptiles	Sparganum (Spirometra spp.)	Various organs
Roundworms (Nematodes):			
Encysted larva (trichina)	Pork; bear meat	Trichinella spiralis Encysted larvae	Small intestine Striated muscle
Infective larva	Snails	Angiostrongylus cantonensis (immature larvae)	Central nervous system
Larvae in muscle	Herring	Heterocheilidae	Larvae in wall of intestine
Flukes (Trematodes):			
Metacercariae in muscle	Fish	Clonorchis sinensis	Liver
Metacercariae	Freshwater fish	Opisthorchis spp.	Liver
Metacercariae	Crabs	Paragonimus westermani	Lungs
Metacercariae in muscle	Marine fish	Heterophyes spp.	Intestine
Metacercariae in muscle	Salmonid fish	Nanophyetus salmincola	Intestine
Protozoa:			
Pseudocysts in animal tissues	Pork; mutton	Toxoplasma gondii	Systemic infection

Thorough cooking and also freezing of the infected meat for several days at temperatures below 0°F. will destroy the parasites and render the meat safe for human consumption. However, the preference of some persons for raw or undercooked beef, pork, and fish, and the eating by others of raw meat from snakes, amphibia and certain invertebrate hosts complicate the problem of preventing human infection.

In 1963 the Agricultural Research Service and the Consumer and Marketing Service of the U. S. Department of Agriculture, Livestock Conservation, Inc., the pork industry, and two business firms decided to investigate cooperatively the possibility of doing something about trichinosis. Their present project is to evaluate the pooled sample digestion technique developed by Dr. W. J. Zimmermann, Iowa State University, Ames, Iowa, for the post-slaughter detection of trichinae in swine in commercial meat-packing establishments. During the first 10 weeks of the project at the Fort Dodge, Iowa, plant of Hormel and Company, 18 trichinous hogs were found among the 157,399 examined by this method. The possible use of this technique for monitoring hams subjected to the dry-cure process in the South has been discussed by the Processed Meat Inspection Division, C&MS.

At the October meeting of the United States Livestock Sanitary Association, New Orleans, Louisiana, a Task Force on Cysticercosis, made up of representatives of agencies of the U. S. Department of Agriculture and the livestock industry, held its first meeting. This Task Force will investigate the possibility of mapping an effective campaign to meet the challenge of increased *Cysticercus bovis* infection in cattle.

It is conceivable that as the production and consumption of fish increase, the problems associated with the parasitic infections of this food source will become more acute and will demand increased efforts of regulatory agencies and the industry to develop methods for their solution.

Many of these problems can be solved by conducting educational programs designed to acquaint the public with specific problems, provide information on methods of preventing infestation and/or reinfestation of ponds and lakes, overcome the consumer's preference for raw or undercooked fish and fish products, and provide wholesome products for his use.

Acknowledgments

The author gratefully acknowledges the very kind assistance of Dr. Glenn L. Hoffman, Parasitologist, Eastern Fish Disease Laboratory, Bureau of Sport Fisheries and Wildlife, Kearnysville, West Virginia, and Dr. Carl J. Sindermann, Director, Tropical Atlantic Biological Laboratory, Bureau of Commercial Fisheries, Fish and Wildlife Service, U. S. Department of the Interior, Miami, Florida, for information on the parasites of food fishes of importance to human health.

MICROBIAL HAZARDS

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The growing number of microbiologists employed by the U. S. Department of Agriculture in recent years reflects a growing concern for the microbial hazards in foods. This concern is also shared by industry. The tendency to control preparation of foods introduces protracted periods of storage, often under conditions favorable for microbial growth and development. It is estimated that only one third of our meals are completely prepared at home. Technology is being pursued to free the housewife of even these remaining chores.

As the interval between preparation and consumption of food increases, the possibility of infections associated with foods also increases. In 1967 over 22,000 cases were reported. Not surprisingly, banquets and school lunches were responsible for the largest number of cases. Salmonellae headed the list followed by *Clostridium perfringens*, and *Staph. aureus*. Various foods have been incriminated, including pork, beef, and other meats; milk; turkey; chicken; and eggs.

Although a large number of food infections were recorded in 1967, there is a feeling that many cases go unreported. There is a need for a better system for reporting outbreaks. The problem is complicated by having to distinguish between toxin producers (*S. aureus* and *Cl. botulinum*) and the true infections (salmonellae and *Cl. perfringens*). A few cases are undoubtedly caused by other groups of organisms which escape identity.

Salmonellosis is the big problem. Detection and identification are long, tedious, and costly. The number of organisms is usually small and they have probably been affected by processing. There is no method available for positive direct identification. This means that these samples have to be preenriched, plated on selective media, and then they must undergo testing for biochemical reactions. Final confirmation is by serology. Each serological type is a species. The whole process will require from 4 to 8 days, which can be prohibitive in this day of limited inventory warehousing. If the food item requires refrigeration, storage before release is obtained can treble the cost and may even preclude the development of certain food types.

Complete absence of salmonellae from foods may be too stringent a requirement and possibly a minimum tolerance will some day be developed. Nevertheless, the requirement has served as a tool to make the industry conscious of microbial hazards in food processing.

TRENDS IN MICROBIAL METHODOLOGY

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The ideal method for linking a microorganism to a food-borne illness will be the one that detects 100 percent of the positives, with no false positives, in zero time. Until then, multiple methods for any given microorganisms will be useful, depending upon the purpose of the examination.

Considerable data have accumulated in recent years on the sources of Clostridium botulinum, and on conditions for toxin production in foods and in pure culture. In almost all of these studies, however, the classical methods developed 30 to 40 years ago for isolation by enrichment in meat infusions and toxin evaluation by mouse protection tests have been used. These methods are relatively simple and rapid and are likely to continue as the methods of choice until some novel approach to identification is developed.

In contrast to botulism, methodology for investigating staphylococcal food poisoning has shifted dramatically in recent years. Isolation media still commonly employ a high NaCl concentration as a selective agent, but tellurite shows considerable promise, and recently a heat-stable DNA-ase has been shown to correlate in most instances with enterotoxin production. Since the DNA-ase can be measured directly in foods, it may provide a rapid screening method for suspected foods. The biggest advance, however, has been in the development of immunological procedures. The recent isolation and purification of specific enterotoxins has led to the development of single and double gel diffusion tests and hemagglutination tests for the direct identification of the enterotoxins. It is now possible to detect the specific enterotoxins in suspected foods as well as to measure their development in culture media.

In salmonellae methodology the primary enrichment media of today contain either selenite or tetrathionate as they did 30 years ago. The most common method for many years has involved enrichment, streaking on a selective medium, and finally isolating colonies for identification by physiological and immunological tests. The major change of the past 6 to 8 years has been the addition of a pre-enrichment broth for examining dried products. Recently, several screening procedures have been developed which shorten the time required, but increase the number of false negatives and positives. These include fluorescent antibody staining, massive inocula for shortened incubation times and greater reliance on presumptive tests. The increasing availability of O and H antisera is increasing the use of group agglutination tests on suspect cultures.

Examining foods for Clostridium perfringens involves selective enrichment to isolate H_2S-producing anaerobes. Recent immunological procedures related to production of a hemolytic toxin (which is not necessarily the cause of enteritis) offer some promise.

Newer approaches to rapid identification include: analysis of cells or early detection of metabolic products by gas chromatography, uptake of radiolabeled substrates, analysis of enzymes produced under standardized conditions by disc electrophoresis, and improved precipitin reactions. None of these, however, has yet been applied on a routine basis.

CHRONIC EFFECTS OF AFLATOXIN IN FARM ANIMAL FEEDING STUDIES*

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Practical feeding trials involving swine and beef cattle have been conducted in order to determine the deleterious effects, if any, of graded levels of aflatoxins in the rations. In addition, analyses of the tissues of these treated animals both chemically and biologically have been performed in order to determine whether any of the aflatoxins in the rations might be transmitted into the edible meat.

One hundred and ten weanling pigs of both sexes were divided into control and treated groups of ten animals per group and fed dietary levels of aflatoxin in the ration ranging from 0 to 810 ppb for a period of 120 days. No evidence of any toxic effects were observed at levels of 233 ppb dietary aflatoxin or below.

Similarly 50 cross-bred Hereford steers were fed 5 dietary levels of aflatoxins ranging from 0 to 1000 ppb for a period of 4-1/2 months. No evidence of any toxic effects were observed at levels of 300 ppb dietary aflatoxin or below

No evidence of transmission of dietary aflatoxin in the ration into edible portions of the swine and cattle was observed at any of the levels studied.

The effects of graded dietary levels of aflatoxin B_1 dispersed in cottonseed meal were studied in dairy cattle for determination of the transmission and production of aflatoxin M_1 in milk. Weekly intake of 67 to 200 mg aflatoxin B_1 produced 70 to 154 ppb aflatoxin M_1 in the lyophilized milk. An important aspect of this study was the finding of complete disappearance of aflatoxin M_1 in the milk after withdrawal of dietary aflatoxin B_1 in a period of 72 hours.

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*This report by A. C. Keyl, A. N. Booth, and M. S. Masri, of the Western Utilization Research and Development Division, and M. R. Gumbmann and W. E. Gagne, of Syntex Laboratories, Palo Alto, California, was also presented at the U. S.-Japan Cooperation in Development of Natural Resources Conference, Honolulu, Hawaii, October 7-11, 1968.

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SOLBERG, M.	New Jersey Agric. Expt. Station	New Brunswick, N. J.
SPIHER, A.T.	Food & Drug Administration, HEW	Washington, D. C.
STEWART, J.H.	Consumer & Marketing Service, USDA	Arlington, Va.
STOEWSAND, G.S.	New York State Agric. Expt. Station	Geneva, N. Y.
SULLIVAN, J.F.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
SULZBACHER, W.L.	Eastern Util. Res. & Dev. Div.	Beltsville, Md.
SUSI, H.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
SWIFT, C.E.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
TALLEY, F.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
THOMPSON, M.P.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
TREADWAY, R.H.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
TUDOR, D.	Rutgers - The State University	New Brunswick, N. J.
VOS, B. J.	Food & Drug Administration, HEW	Washington, D. C.

LIST OF ATTENDANCE - continued

Name	Organization	Address
WAZENSKI, T.	Food & Drug Administration, HEW	Philadelphia, Pa.
WEESE, S. J.	West Virginia Agric. Expt. Station	Morgantown, W. Va.
WELLS, P. A.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
WHITMORE, R. A.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
WICKROSKI, A.	The Connecticut Agric. Expt. Station	New Haven, Conn.
WINDUS, W.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
WITNAUER, L.P.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
WOODMANSEE, C.W.	Delaware Agric. Expt. Station	Newark, Del.
WOODWARD, C.F.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
WRIGLEY, A. N.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
ZITTLE, C.A.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.

This conference on food safety was one of a series of annual collaborators' conferences organized by the regional utilization divisions of the Agricultural Research Service, U. S. Department of Agriculture. The collaborators are staff representatives of the State Agricultural Experiment Stations in each of the four regions. To assure depth and breadth in subject matter, a single area of important research is selected for each conference.